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BIOLOGICAL BULLETIN

A CASE OF NORMAL IDENTICAL QUADRUPLETS IN THE NINE-BANDED ARMADILLO, AND ITS BEARING ON THE PROBLEMS OF IDENTICAL TWINS AND OF SEX DETERMINATION.[1]

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In a former contribution from this laboratory by A. M. Spurgin, M.D., occurs the following statement: "A year ago, Dr. W. M. Wheeler, of the School of Zoölogy, had the good fortune to secure four embryos of the *Dasypus novemcinctus* from an adult female which had been kept in the laboratory for several weeks. . . . He found four placentæ inclosed in one amnion, but has not since had time to study the subject further."

When the writers arrived in Austin last September they made arrangements to obtain material with which further to investigate this suggestive problem, and were fortunate enough to enlist the services of a naturalist living in a part of Texas where the armadillo abounds. In this way we have been able to secure several gravid females, from a study of which a number of interesting facts have been brought to light.

There are always four embryos, corresponding in number to the two pairs of mammæ, which are thoracic and abdominal in position. The embryos are not enclosed in one amnion, but each has its own distinct amniotic envelope, the cavity of which does not communicate with those of the adjacent amnia. This was proved by the experiment of inflating one amnion and noting that the air did not pass to the cavities of the contiguous amnia. The four amnia, however, are enclosed in a single chorionic vesicle.

[1] Contribution from the Zoölogical Laboratory of the University of Texas, No. 98.

[2] *American Journal of Anatomy*, Vol. III., No. 1.

An examination of the external villous layer of the chorion shows that placentation is unique in that areas of villi, although apparently forming a complete zone about the equatorial region of the uterine wall, are in reality arranged in four closely approximated ovoid areas, two large and lateral in position, and two smaller, one dorsal and the other ventral. The proximal (*i. e.*, the posterior or vaginal) and distal ends of the chorionic vesicle are practically free of villi, except at each pole, where the presence of a small tuft of villi causes the chorion to adhere very firmly to the uterine wall (see Fig. 1). The villous zone, composed as it is of four ovoid areas, extends into the polar regions in the form of four scallops at each end, and on the amniotic side of the four scallops are situated the points of attachment of the four umbilical cords (see Fig. 2).

In the advanced stages of gestation the chorionic vesicle is shaped very much like a football, and the four lines on the inside wall, along which the adjacent amnia meet, correspond to the seams of the ball (see Fig. 1). In the young stages, however, the chorion is in the shape of an octaedron with rounded edges, and flattened dorso-ventrally, and has its entire surface covered with villi (see Fig. 3). From this we may conclude that the definitive condition of the placenta has been attained by a localization of the villi into ovoid areas, each of which is to be looked upon as the vascular center for an umbilicus.

The points of attachment of the umbilical cords lie close to the amniotic partitions, and when the chorion is viewed from the distal end the cord in each case is seen to be situated just to the right of the partition (see Fig. 2). In embryos about one fourth grown the partition between any two contiguous embryos may be easily separated into its component parts, but in older stages the fusion of the membranes is so complete that separation is impossible. In late stages, therefore, the chorionic cavity is completely divided longitudinally into four separate compartments, each of which contains an embryo. Although the portions of the chorionic wall that lie between the successive amniotic partitions are in the form of quadrants, yet they do not coincide with the ovoid areas; for the partitions do not meet the chorionic wall in lines corresponding to the divisions of the ovoid

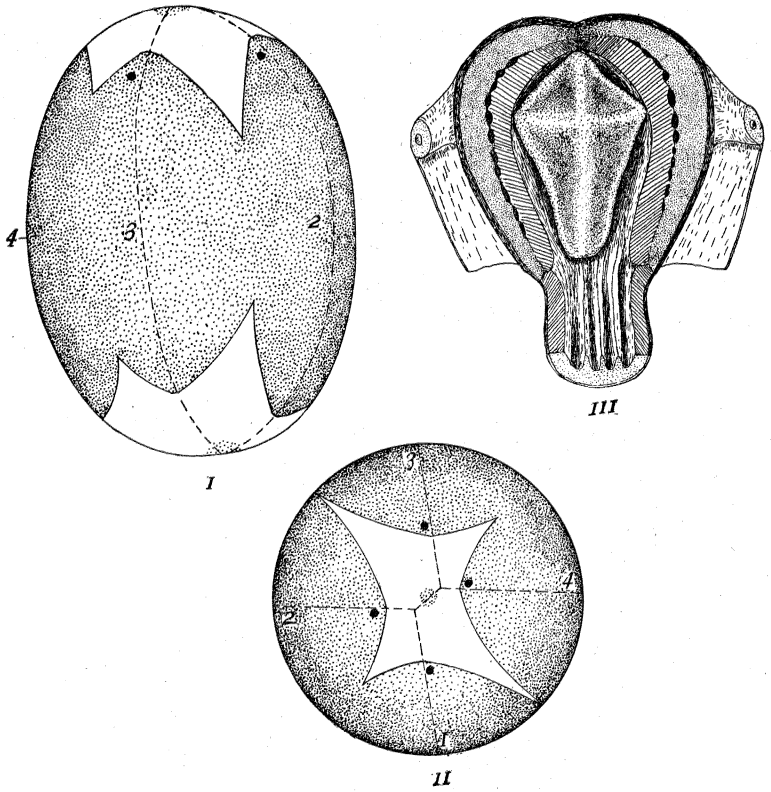

FIG. 1. A diagrammatic representation of an approximately dorsal view of a chorionic vesicle taken from the uterus about two weeks (estimated) before the young would have been born. Three of the ovoid areas are in view, and the broken lines represent the lines along which the amniotic partitions meet the inside wall of the chorion. The clear areas at the ends are broken into by the scallops of the ovoid areas. In this, as in the succeeding figure, the points of attachment of the umbilical cords are indicated by large dots. Note especially that the indentations between the scallops of areas 3 and 4 are much shallower than those between areas 2 and 3, leaving a broader connection between the former than between the latter. This is an indication that embryos 3 and 4, located respectively on dorsal and left lateral areas (similarly embryos 1 and 2, located on the ventral and right lateral areas), are natural pairs. For the significance of this arrangement see table and text. One half natural size.

FIG. 2. A view of the distal end of the preceding figure. This is introduced to show the relation existing between the amniotic partitions within the chorionic cavity. The figure also brings out the fact that the embryos may be paired, together with the ovoid areas to which they are attached by the umbilical cords. One half natural size.

FIG. 3. This shows a chorionic vesicle *in situ*, as revealed by splitting open the uterus along the mid-ventral line. The age of this vesicle is estimated at one month. Unfortunately the splitting was done before the specimen reached our hands, and extended so deep as to divide the vesicle into two parts. The parts, however, were well preserved *in situ*, and the reconstruction could be made with certainty. Note that the vesicle is octaedronal in shape, and that its entire surface is covered with villi. Natural size.

areas, but each partition passes through the middle of an area and thus intersects the apices of the proximal and distal scallops (see Fig. 1). The four partitions, furthermore, do not meet within the cavity in a line corresponding to the polar axis of the vesicle, but are fused in such a way that the cavities containing the embryos numbered 1 and 3 meet on their inner faces, while those containing 2 and 4 do not (see Fig. 2).

In a note in the current issue of *Science* (April 30) Professor H. H. Lane gives a brief account of placentation in the armadillo that differs in a number of particulars from the above. The discrepancies might be explained by the fact that Lane's account rests upon observations made upon a single deciduate placenta, taken after the birth of a litter. In addition to its incompleteness, his account differs from that offered here chiefly in locating the points of attachment of two of the umbilical cords on each of the large "disc-shaped areas," and none on the smaller areas. This condition is certainly not found in any of our material. A more fundamental difference between the two accounts lies in the interpretation of the condition of the chorion, Lane holding an opinion completely out of accord with the facts here presented, that the definitive chorion is the product of a complete fusion of four chorionic vesicles into one.

The unique interest of this case lies in the fact that the four embryos of a given set are not only invariably of the same sex, but are practically identical in many other respects. Measurements of the body and head lengths showed no essential differences between the individuals of a set of quadruplets, but a more searching comparison, consisting of carefully confirmed counts of the total number of plates in the nine bands of the armor, revealed, as one might expect, slight departures from complete identity. The results obtained from the examination of two sets will serve to indicate the small range of these departures.

Set A (Females).	Set B (Males).
No. 1.....556 (+ 1) plates[1]	No. 1.....571 (+ 2) plates
No. 2.....555 (+ 2) "	No. 2.....573 (+ 1) "
No. 3.....553 (+ 2) "	No. 3..... ..569 "
No. 4..... .....551 (+ 4) "	No. 4.....568 "

[1] The numbers enclosed in parentheses refer to certain rudimentary plates that are more or less united with other plates. It is impossible to tell in the embryos whether

In both cases the range of variation is five, or less than one per cent. The difference in totals between the two sets is very much greater — a difference that does not indicate a sexual dimorphism, for an examination of five unrelated specimens of both sexes that happen to be on hand, shows a range of from 547 to 575 plates, a variation nearly six times as great as that existing between a given set of quadruplets.

This very marked similarity among the individuals of a given set suggests that we have here a condition showing a degree of identity that, in all probability, is at least as close as that found in the well-known case of "true" or "identical twins" in the human race, where it is supposed that each embryo arises from from one of the two blastomeres of the two-cell stage. In the case of *Dasypus*, each embryo probably arises from one of the blastomeres of the four-cell stage.

This immediately turns our attention to a further consideration of the fœtal membranes, the amnion and the chorion, and of the manner in which they arise. It was stated above that each embryo is enclosed in a separate amnion, and that the four amnia are within a single chorion. We say single chorion advisedly, because its surface gives no indication of being a multiple structure, that is, the product of the fusion of four chorionic vesicles at an earlier period, as suggested by Lane; nor do the facts revealed by a study of sections of the chorionic wall seem to bear out such an interpretation. One must admit, however, the great difficulty of any attempt at a correct interpretation of the exact relationship existing between the fœtal membranes as seen in these advanced stages. The solution of that problem must be sought in a study of young stages, when the membranes are in process of formation.

To have four ova ripen, be fertilized, and reach the uterus at the same time, and always arrange themselves in a definite fashion

or not these small plates would later have become separated off to form distinct ones, but we are inclined to believe that such is the case, for in the adult armor there appear to be no signs of double plates. If we count these rudimentary plates as complete plates, then embryos 1 and 2 (Set A) would have exactly the same number of plates, as would also embryos 3 and 4. The numbers of plates in the corresponding embryos of Set B, although not identical, differ to the minimum extent, one plate in each case.

with reference to one another and to the uterine wall, would be in itself a remarkable coincidence. The chief difficulty, however, in the way of accepting the view that the chorionic vesicle is a multiple structure lies not so much in the interpretation of the relation existing between the fœtal membranes, nor in the discovery of the mechanics involved in the attainment of such a relation, as in the explanation of the fact that the embryos of any given set are always practically identical and of the same sex. At first sight it might seem that this identity could be explained by the fact that the embryos are apparently under the same environmental influences, inasmuch as they are enclosed within a single chorion. But in reality they are not under exactly the same influences, for each embryo has its own amnion, and is, therefore, surrounded by a fluid apparently as distinct as though each were in a separate chorion. This forces us to the conviction that the four embryos of a set are derived from a single egg.

If this is the case, one can only conjecture as to the manner in which the conditions seen in the older stages have arisen, and any suggestions which we may have to offer at this time must be taken as tentative. It may be supposed, for example, that the developing egg, after it has reached a stage corresponding to that of an inner-cell mass and trophoblast in other mammals, has, in the case of *Dasypus*, four inner-cell masses — a cell mass for each quadrant; and furthermore that the cells of any given inner-cell mass, together with the trophoblastic cells of its quadrant, are the lineal descendants of one of the blastomeres of the four-cell stage. This possible interpretation receives a striking confirmation in the fact that the four embryos can be arranged into two pairs, the individuals of which approach almost complete identity; and these identicals are not only adjacent to each other, but are also attached to placental areas that are closely united (see Figs. 1 and 2 and table). If all four embryos are derived from a single egg, this is exactly what we should expect to find; for surely the two individuals derived from one of the blastomeres of the two-cell stage ought to be more nearly similar to each other than to the individuals of the other blastomere. If the above be granted, it is an easy matter to explain the origin of the conditions found in late stages; and the condition of a

single chorion with four amnia would receive a rational explanation.

We are, of course, aware that other possible interpretations might be given, such as has been offered from time to time to account for human identical twins. Our studies of the ovarian tissues of *Dasypus* have excluded the most plausible of these, viz., that the ovum may have more than one germinal vesicle. Further investigation will have to settle the next most plausible explanation offered, that the polar bodies may become functional. None of the other suggestions found in the literature seems as credible as the view to which we incline, and in support of our contention we have facts derived from an examination of two sets of ovaries from gravid females. In one set there is but a single corpus luteum, and, while in the other there are two corpora lutea, one has practically disappeared, and evidently had been formed during a previous pregnancy.

After all, the development of the fœtal membranes is a matter of secondary interest, as compared with the more fundamental problem of the identity of the embryos of a set, and its corollary that of sex determination. The bearing of this work on the latter problem is obvious, and we hope that a study of the early developmental stages will lend a solution to this problem, and also furnish a satisfactory explanation of the puzzling question of "identical twins"; and thus raise this explanation from the plane of conjecture to the dignity of observed fact.

AUSTIN, TEXAS,
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